

REC'D 09 JUN 2004

WIPO

PCT

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

June 04, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM
THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK
OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE.

APPLICATION NUMBER: 60/463,776

FILING DATE: April 18, 2003

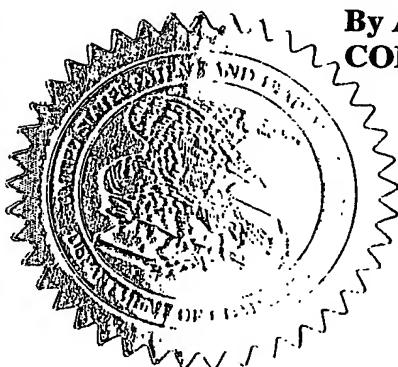
RELATED PCT APPLICATION NUMBER: PCT/US04/11473

BEST AVAILABLE COPY

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS



E. BORNETT
Certifying Officer



PRIORITY
DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

04-21-03

60463776 04/18/03

SUBSTITUTE for Provisional Application for Patent Cover Sheet PTO/SB/16 (10-01)
 Approved for use through 10/31/2002 OMB 0651-0032
 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

04/18/03
 JC698 U.S. PTO

1033 U.S. PRO
 6046376 04/18/03

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

DOCKET NUMBER 21198PV

INVENTOR(S)					
Given Name (first and middle if any)		Family Name or Surname	Residence (City and either State or Foreign Country)		
Hideki Carina P. Andrew D.		Sano Tan Howard	Rahway, New Jersey 07065 Metuchen, New Jersey 08840 Park Ridge, New Jersey 07656		
<input type="checkbox"/> Additional inventors are being named on the separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
RHESUS MONKEY BOMBESIN RECEPTOR SUBTYPE-3 (BRS-3), NUCLEOTIDES ENCODING SAME, AND USES THEREOF					
CORRESPONDENCE ADDRESS					
Direct all Correspondence to:		Merck & Co., Inc. Patent Department - RY60-30 P.O. Box 2000 Rahway			
		<input checked="" type="checkbox"/> Customer Number 000210			
STATE	New Jersey	ZIP CODE	07065	COUNTRY	U.S.A.
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification	Number of Pages	29	<input type="checkbox"/> CD(s), Number		
<input checked="" type="checkbox"/> Drawing(s)	Number of Sheets	8	<input type="checkbox"/> Other (specify)		
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees					
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 13-2755				FILING FEE AMOUNT (\$)	\$160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

No.

Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE Alysia A. Finnegan
 TYPED or PRINTED NAME Alysia A. Finnegan
 TELEPHONE (732)594-2583

Date 04/18/2003

REGISTRATION NO. 48,878

EXPRESS MAIL CERTIFICATE	
DATE OF DEPOSIT	April 18, 2003
EXPRESS MAIL NO.	EL920763664US
I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS EXPRESS MAIL "POST OFFICE TO ADDRESSEE" ON THE ABOVE DATE IN AN ENVELOPE ADDRESSED TO ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231.	
MAILED BY	<u>Core Schepke</u> DATE 4-18-03

In Duplicate

TITLE OF THE INVENTION

RHESUS MONKEY BOMBESIN RECEPTOR SUBTYPE-3 (BRS-3),
NUCLEOTIDES ENCODING SAME, AND USES THEREOF

5 FIELD OF THE INVENTION

The present invention relates to rhesus monkey bombesin receptor subtype-3, herein designated rhBRS-3, to isolated nucleic acid molecules which encode this receptor, to recombinant vectors and hosts comprising DNA encoding this receptor and to use of rhBRS-3 in various assays.

10

BACKGROUND OF THE INVENTION

Bombesin, a tetradecapeptide originally isolated from frog skin, represents the first member of a large family of regulatory peptides named bombesin-like peptides. Two bombesin-like peptides, gastrin-releasing peptide (GRP) 15 (McDonald *et al.*, *Biochem. Biophys. Res. Commun.* 90: 227-33 (1979)) and neuromedin B (NMB) (Minamino *et al.*, *Biochem. Biophys. Res. Commun.* 114: 541-548 (1983)) have been found in mammals.

Bombesin receptor subtype-3 (BRS-3, also named BB3) is one of three subtypes of bombesin receptors, which was identified based on its high degree of 20 homology to mammalian bombesin receptors. BRS-3 is a member of the G protein-coupled receptor superfamily and has been cloned from human, mouse and sheep (Whitley *et al.*, *J. Mol. Endocrinol.* 23: 107-16 (1999)). A naturally occurring high affinity ligand for BRS-3 has not been identified. However, a synthetic peptide, [D-Tyr⁶-betaAla¹¹-Phe¹³-Nle¹⁴] bombesin(6-14) (hereinafter dYB) was shown to have 25 high affinity for all three human bombesin receptor subtypes (Pradhan *et al.*, *Eur. J. Pharmacol.* 343: 275-87 (1998)).

The human BRS-3 sequence was originally described by Fathi *et al.* (*J. Biol. Chem.* 268(8): 5979- 84 (1993)). A variant of the human sequence was also described (U.S. Patent No. 6,143,521).

30 In addition to the human clones described above, rat BRS-3 sequences were disclosed by Liu *et al* (WO 03/014310) and by Spindel *et al.* (U.S. Patent No.

5,656,749). A truncated BRS-3 was also isolated from rat by Lane et al. (WO 01/10889).

Bombesin, bombesin-like peptides and related receptors participate in a diverse array of physiological processes. BRS-3 has been implicated in the 5 regulation of neuroendocrine function and energy metabolism (Ohki *et al. Nature* 390: 165-69 (1997)). Mice lacking functional BRS-3 are hyperphagic and have a reduced metabolic rate, which leads to the development of obesity, hypertension and diabetes as adults. Additionally, bombesin-like peptides may contribute to the pathogenesis of some human carcinomas (For review, see Lebacq-Verheyden et al., in 10 *Handbook of Experimental Pharmacology*, Sporn, M.N. and Roberts, A.B., eds., Vol. 95, pp. 71-124, Springer-Verlag, Berlin).

Despite the identification of the cDNA clones encoding bombesin receptor subtypes mentioned above, it would be advantageous to identify additional 15 mammalian genes encoding bombesin receptor subtypes in order to allow screening to identify novel bombesin receptor modulators that may contribute to the regulation of endocrine processes, metabolism, or the cell cycle.

SUMMARY OF THE INVENTION

The present invention relates to an isolated or purified nucleic acid 20 molecule (polynucleotide) which encodes a novel rhesus monkey bombesin receptor subtype-3 (hereinafter rhBRS-3). The DNA molecules disclosed herein may be transfected into a host cell of choice wherein the recombinant host cell provides a source for substantial levels of an expressed functional rhBRS-3 protein (SEQ ID NO:2). This receptor protein provides a screening target to identify modulators of 25 bombesin and bombesin-like peptides, which may be involved in the pathogenesis of a variety of human disorders when deregulated.

The present invention also relates to isolated nucleic acid molecules comprising a sequence of nucleotides that encode a rhesus monkey BRS-3 protein as set forth in SEQ ID NO:2. In an exemplary embodiment of this aspect of the 30 invention, the nucleic acid molecule comprises a sequence of nucleotides as set forth in SEQ ID NO:1.

Included in this invention are biologically active fragments or mutants of SEQ ID NO:1, which encode mRNA expressing a novel rhBRS-3 protein. Any such biologically active fragment and/or mutant will encode either a protein or protein fragment which at least substantially mimics the pharmacological properties 5 of the rhBRS-3 protein, including but not limited to the rhBRS-3 protein as set forth in SEQ ID NO:2.

The present invention further relates to a process for expressing a rhesus monkey BRS-3 protein in a recombinant host cell, comprising: (a) introducing a vector comprising an isolated nucleic acid molecule into a suitable host cell, the 10 nucleic acid molecule comprising a sequence of nucleotides that encodes a rhesus monkey BRS-3 protein as set forth in SEQ ID NO:2; and, (b) culturing the host cell under conditions which allow expression of said rhesus monkey BRS-3 protein.

The present invention also relates to recombinant vectors and recombinant host cells, both prokaryotic and eukaryotic, which contain the nucleic 15 acid molecules disclosed throughout this specification.

Another aspect of the present invention is a substantially purified form of a rhesus monkey BRS-3 protein which consists of the amino acid sequence disclosed in FIGURE 2 (SEQ ID NO:2). Characterization of the BRS-3 protein will allow for screening to identify novel bombesin receptor subtype-3 modulators that 20 may have a role in the regulation of endocrine processes or metabolism. As noted above, heterologous expression of rhesus monkey BRS-3 disclosed herein is contemplated at levels substantially above endogenous levels and will allow for the pharmacological analysis of compounds which may contribute to the pathogenesis of a variety of human disorders associated with deregulated BRS-3 expression. 25 Heterologous cell lines expressing a functional rhesus monkey BRS-3 (e.g., functional forms of SEQ ID NO: 2), can be used to establish functional or binding assays to identify novel BRS-3 modulators that may be useful in the development of therapeutics for human diseases associated with deregulated BRS-3 expression.

30 The present invention also provides biologically active fragments and/or mutants of a rhesus monkey BRS-3 protein, comprising the amino acid

sequence as set forth in SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic or prophylactic use and would be useful for

5 screening for selective modulators, including but not limited to agonists and/or antagonists for rhesus monkey bombesin and bombesin-like peptide receptor pharmacology.

10 The present invention also relates to a substantially purified, fully processed (including proteolytic processing, glycosylation and/or phosphorylation), mature BRS-3 protein obtained from a recombinant host cell containing a DNA expression vector comprising nucleotide sequence as set forth in SEQ ID NO:1, which expresses the rhBRS-3 protein.

15 The present invention also relates to rhesus monkey BRS-3 fusion constructs, including but not limited to fusion constructs which express a portion of the rhesus monkey BRS-3 protein linked to various markers, including but in no way limited to GFP (Green fluorescent protein), the MYC epitope, GST, and Fc. Any such fusion constructs may be expressed in the cell line of interest and used to screen for modulators of the rhesus monkey BRS-3 protein disclosed herein.

20 The present invention further relates to methods of expressing rhesus monkey BRS-3 proteins and biological equivalents disclosed herein, assays employing these gene products, recombinant host cells which comprise DNA constructs which express these proteins, and compounds identified through these assays which act as agonists or antagonists of BRS-3 activity.

25 The present invention further relates to methods for screening for compounds which modulate the expression of DNA or RNA encoding a rhBRS-3 protein as well as compounds which effect the function of the rhBRS-3 protein.

30 Also provided herein is a method for identifying compounds that modulate rhesus monkey bombesin receptor subtype-3 expression, comprising contacting a test compound with rhesus monkey bombesin receptor subtype-3, and

determining whether the test compound interacts with rhesus monkey bombesin receptor subtype-3.

This invention further relates to a method for determining whether a substance is capable of binding to rhesus monkey BRS-3 (rhBRS-3) comprising: (a) 5 providing test cells by transfecting cells with an expression vector that directs the expression of rhBRS-3 in the cells; (b) exposing the test cells to the substance; (c) measuring the amount of binding of the substance to rhBRS-3; and, (d) comparing the amount of binding of the substance to rhBRS-3 in the test cells with the amount of binding of the substance to control cells that have not been transfected with rhBRS-3.

10

Also provided herein is a method for determining whether a substance is a potential agonist or antagonist of rhBRS-3 comprising: (a) transfecting or transforming cells with an expression vector that directs expression of rhBRS-3 in the cells, resulting in test cells; (b) allowing the test cells to grow for a time sufficient to 15 allow rhBRS-3 to be expressed; (c) exposing the cells to a labeled ligand of rhBRS-3 in the presence and in the absence of the substance; and, (d) measuring the binding of the labeled ligand to rhBRS-3; where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of rhBRS-3.

20

Another preferred aspect of the present invention is a substantially purified membrane preparation, partially purified membrane preparation, or cell lysate which has been obtained from a recombinant host cell transformed or transfected with a DNA expression vector which comprises and appropriately 25 expresses a complete open reading frame as set forth in SEQ ID NO:1, resulting in a functional form of rhBRS-3.

As used throughout the specification and in the appended claims, the singular forms "a," "an," and "the" include the plural reference unless the context 30 clearly dictates otherwise.

As used throughout the specification and appended claims, the following definitions and abbreviations apply:

"Substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. As used interchangeably, the terms "substantially free from other nucleic acids," "substantially purified," "isolated nucleic acid" or "purified nucleic acid" also refer to DNA molecules which comprise a coding region for a rhesus monkey BRS-3 protein that has been purified away from other cellular components. Thus, a rhesus monkey BRS-3 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, 5 preferably no more than 5%, more preferably no more than 1%, and even more 10 preferably no more than 0.1%, of non-rhesus BRS-3 nucleic acids. Whether a given rhesus monkey BRS-3 DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, 15 ethidium bromide staining, or by sequencing.

"Substantially free from other proteins" or "substantially purified" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a rhesus monkey BRS-3 protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, 20 no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-rhesus monkey BRS-3 proteins. Whether a given rhesus monkey BRS-3 protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis 25 (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting. As used interchangeably, the terms "substantially free from other proteins" or "substantially purified", or "isolated rhesus monkey BRS-3 protein" or "purified rhesus monkey BRS-3 protein" also refer to rhesus monkey BRS-3 protein that has been isolated from a natural source.

30 Use of the term "isolated" or "purified" indicates that rhesus monkey BRS-3 protein has been removed from its normal cellular environment. Thus, an

isolated rhesus monkey BRS-3 protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated rhesus monkey BRS-3 protein is the only protein present, but instead means that an isolated rhesus monkey BRS-3 protein is 5 substantially free of other proteins and non-amino acid material (e.g., nucleic acids, lipids, carbohydrates) naturally associated with the rhesus BRS-3 protein *in vivo*. Thus, a rhesus monkey BRS-3 protein that is recombinantly expressed in a prokaryotic or eukaryotic cell and substantially purified from this host cell which does not naturally (i.e., without intervention) express this BRS-3 protein is of course 10 "isolated rhesus monkey BRS-3 protein" under any circumstances referred to herein.

As noted above, a rhesus BRS-3 protein preparation that is an isolated or purified rhesus monkey BRS-3 protein will be substantially free from other proteins and will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more 15 preferably no more than 0.1%, of non-rhesus monkey BRS-3 proteins.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar 20 residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid).

The term "rhBRS" refers to a -rhesus monkey bombesin receptor subtype-3--

The term "mammalian" will refer to any mammal, including a human 25 being.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the nucleotide sequence of rhesus monkey BRS-3 cDNA, as set forth in SEQ ID NO:1.

30 FIGURE 2 shows the predicted amino acid sequence of rhesus monkey BRS-3 protein, as set forth in SEQ ID NO:2.

FIGURE 3 shows an alignment of the human (SEQ ID NO:17, *see* Fathi et al., *supra*), rat (SEQ ID NO:18, *see* Liu et al., WO 03/014310) and rhesus monkey (SEQ ID NO:1) BRS-3 nucleotide sequences. Nucleotides that are different among the BRS-3 sequences are shown in bold. Dashes indicate that spaces were added to facilitate the alignment. A consensus sequence (SEQ ID NO:19), derived by comparing the above nucleotide sequences, is also shown.

FIGURE 4 shows an alignment of the human (SEQ ID NO:20, *see* Fathi et al., *supra*), rat (SEQ ID NO:21, *see* Liu et al., WO 03/014310) and rhesus monkey (SEQ ID NO:2) BRS-3 open reading frames. Amino acids that are different among the BRS-3 sequences are shown in bold. Dashes indicate that spaces were added to facilitate the alignment. A consensus sequence (SEQ ID NO:22), derived by comparing the above protein sequences, is also shown.

DETAILED DESCRIPTION OF THE INVENTION

30 The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes rhesus monkey bombesin receptor subtype-3 protein (SEQ ID NO:2). A preferred aspect of this portion of the present invention is

disclosed in FIGURE 1 (SEQ ID NO:1), which shows a DNA molecule encoding a novel rhBRS-3 protein (SEQ ID NO:2).

The isolated nucleic acid molecules of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and 5 complementary DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecule of the present invention may also include a ribonucleic acid molecule (RNA). For most cloning purposes, DNA is a preferred nucleic acid. The nucleic acid molecules of the present invention are 10 substantially free from other nucleic acids.

As noted above, an exemplary embodiment of the present invention is an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel rhesus monkey bombesin receptor subtype-3 protein, this DNA 15 molecule comprising the nucleotide sequence disclosed herein as SEQ ID NO:1. This rhBRS-3 nucleic acid molecule was identified through RT-PCR as described in detail in EXAMPLE 1.

Included in this invention are biologically active fragments or mutants of SEQ ID NO:1, which encode mRNA expressing a novel rhBRS-3 protein. Any 20 such biologically active fragment and/or mutant will encode either a protein or protein fragment which at least substantially mimics the pharmacological properties of the rhBRS-3 protein, including but not limited to the rhBRS-3 protein as set forth in SEQ ID NO:2. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and 25 carboxy-terminal truncations such that these mutations encode mRNA which express a functional rhBRS-3 protein in a eukaryotic cell, such as *Xenopus* oocytes, so as to be useful for screening for agonists and/or antagonists of rhesus monkey BRS-3 activity.

30 In preferred embodiments of the invention, DNA is ligated into a vector, and introduced into suitable host cells to produce transformed cell lines that

express the rhesus monkey BRS-3 protein, or a fragment thereof. The resulting cell lines can then be produced in quantity for reproducible quantitative analysis of the effects of drugs on receptor function.

In other embodiments, mRNA may be produced by *in vitro* transcription of DNA encoding the invention peptide. This mRNA can then be injected into *Xenopus* oocytes where the RNA directs the synthesis of the rhesus monkey BRS-3 protein. Alternatively, the invention-encoding DNA can be directly injected into oocytes for expression of a functional invention peptide. The transfected mammalian cells or injected oocytes may then be used in the methods of drug screening provided herein.

Therefore, the heterologous expression of the rhesus monkey BRS-3 protein will allow the pharmacological analysis of compounds that may contribute to the regulation of the endocrine system, cell cycle or metabolism. Heterologous cell lines expressing these rhBRS-3 proteins can be used to establish functional or binding assays to identify novel rhBRS-3 modulators that may be useful in the development of novel human therapeutics for diseases related to deregulated bombesin receptor expression.

Another aspect of the present invention is a substantially purified form of a rhesus monkey BRS-3 protein which consists of the amino acid sequence disclosed in FIGURE 2 (SEQ ID NO:2). This receptor protein provides a screening target to identify modulators of bombesin and bombesin-like peptides, which may be involved in the pathogenesis of a variety of human disorders when deregulated.

The present invention also provides biologically active fragments and/or mutants of a rhesus monkey BRS-3 protein, comprising the amino acid sequence as set forth in SEQ ID NO:2, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic or prophylactic use and would be useful for screening for selective modulators, including but not limited to agonists and/or

antagonists for rhesus monkey bombesin and bombesin-like peptide receptor pharmacology.

The present invention also relates to a substantially purified, fully processed (including proteolytic processing, glycosylation and/or phosphorylation),

5 mature BRS-3 protein obtained from a recombinant host cell containing a DNA expression vector comprising nucleotide sequence as set forth in SEQ ID NO:1, which expresses the rhBRS-3 protein. It is especially preferred that the recombinant host cell be a eukaryotic host cell, such as a mammalian cell line, or *Xenopus* oocytes, as noted above.

10 As noted above, a preferred aspect of the present invention is disclosed in FIGURE 2 (SEQ ID NO:2), which indicates the amino acid sequence of the rhesus monkey BRS-3 protein of the present invention. Characterization of this protein will allow for screening to identify novel bombesin receptor subtype-3 modulators that may have a role in the regulation of endocrine processes or metabolism.

15 Heterologous expression of rhesus monkey BRS-3 disclosed herein is contemplated at levels substantially above endogenous levels and will allow for the pharmacological analysis of compounds which may contribute to the pathogenesis of a variety of human disorders associated with deregulated BRS-3 expression. Heterologous cell lines expressing a functional rhesus monkey BRS-3 (e.g., functional forms of SEQ ID

20 NO: 2), can be used to establish functional or binding assays to identify novel BRS-3 modulators that may be useful in the development of therapeutics for human diseases associated with deregulated BRS-3 expression.

25 The rhesus monkey BRS-3 receptor proteins of the present invention may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

30 Accordingly, the present invention relates to rhesus monkey BRS-3 fusion constructs, including but not limited to fusion constructs which express a portion of the rhesus monkey BRS-3 protein linked to various markers, including but

in no way limited to GFP (Green fluorescent protein), the MYC epitope, GST, and Fc. Any such fusion constructs may be expressed in the cell line of interest and used to screen for modulators of the rhesus monkey BRS-3 protein disclosed herein.

5 This invention also relates to various functional domains of the rhBRS-3 receptor, such as the extracellular domain and the intracellular domain, and to hybrid molecules comprising at least one of these sequences. Accordingly, the present invention includes chimeric polypeptides wherein at least one domain of the rhesus monkey BRS-3 polypeptide is linked a non-rhesus monkey BRS-3 sequence of 10 amino acid residues to produce a chimeric polypeptide. The present invention also includes isolated nucleic acid molecules, comprising a sequence of nucleotides that encodes said chimeric polypeptide.

15 As noted above, a preferred aspect of this invention is a DNA molecule described in FIGURE 1 as rhesus monkey BRS-3 and set forth as SEQ ID NO:1, which encodes the rhesus monkey bombesin receptor subtype-3 protein shown in FIGURE 2 and set forth as SEQ ID NO:2.

20 It is well understood in the art that differing DNA molecules may express an identical protein due to codon redundancy. Accordingly, this invention also relates to synthetic DNA that encodes the rhBRS-3 protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ ID NO:1 but still encodes the same rhBRS-3 protein as SEQ ID NO:2. Such synthetic DNAs are intended to be within the scope of the present invention. If it is desired to express such synthetic DNAs in a particular host cell or organism, the 25 codon usage of such synthetic DNAs can be adjusted to reflect the codon usage of that particular host, thus leading to higher levels of expression of the BRS-3 protein in the host.

30 Therefore, the present invention discloses codon redundancy that may result in differing DNA molecules expressing an identical protein. For purposes of this specification, a sequence bearing one or more replaced codons will be defined as a degenerate variation. Also included within the scope of this invention are mutations

either in the DNA sequence or the translated protein that do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in the functionality of the polypeptide.

5

It is known that DNA sequences coding for a peptide may be altered so as to code for a peptide that has properties that are different than those of the naturally occurring peptide. Methods of altering the DNA sequences include but are not limited to site directed mutagenesis. Examples of altered properties include but 10 are not limited to changes in the affinity of an enzyme for a substrate or receptor for a ligand.

Any of a variety of procedures may be used to clone rhBRS-3. These methods include, but are not limited to, (1) a RACE PCR cloning technique 15 (Frohman, et al., 1988, *Proc. Natl. Acad. Sci. USA* 85: 8998-9002). 5' and/or 3' RACE may be performed to generate a full-length cDNA sequence. This strategy involves using gene-specific oligonucleotide primers for PCR amplification of rhBRS-3 cDNA. These gene-specific primers are designed through identification of an expressed sequence tag (EST) nucleotide sequence which has been identified by 20 searching any number of publicly available nucleic acid and protein databases; (2) direct functional expression of the rhBRS-3 cDNA following the construction of a rhBRS-3-containing cDNA library in an appropriate expression vector system; (3) screening an rhBRS-3-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled degenerate oligonucleotide probe designed from 25 the amino acid sequence of the rhBRS-3 protein; (4) screening an rhBRS-3-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding the rhBRS-3 protein. This partial cDNA is obtained by the specific PCR amplification of rhBRS-3 DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence known for other 30 kinases which are related to the rhBRS-3 protein; (5) screening a rhBRS-3-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector

with a partial cDNA or oligonucleotide with homology to a mammalian rhBRS-3 protein. This strategy may also involve using gene-specific oligonucleotide primers for PCR amplification of rhBRS-3 cDNA identified as an EST as described above; or (6) designing 5' and 3' gene specific oligonucleotides using SEQ ID NO:1 as a

5 template so that either the full-length cDNA may be generated by known RACE techniques, or a portion of the coding region may be generated by these same known RACE techniques to generate and isolate a portion of the coding region to use as a probe to screen one of numerous types of cDNA and/or genomic libraries in order to isolate a full-length version of the nucleotide sequence encoding rhBRS-3.

10 It is readily apparent to those skilled in the art that other types of libraries, as well as libraries constructed from other cell types-or species types, may be useful for isolating a rhBRS-3 -encoding DNA or a rhBRS-3 homologue. Other types of libraries include, but are not limited to, cDNA libraries derived from other cells.

15 It is readily apparent to those skilled in the art that suitable cDNA libraries may be prepared from cells or cell lines which have rhBRS-3 activity. The selection of cells or cell lines for use in preparing a cDNA library to isolate a cDNA encoding rhBRS-3 may be done by first measuring cell-associated rhBRS-3 activity using any known assay available for such a purpose.

20 Preparation of cDNA libraries can be performed by standard techniques well known in the art. Well known cDNA library construction techniques can be found for example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. Complementary DNA libraries may also be obtained from numerous commercial

25 sources, including but not limited to Clontech Laboratories, Inc. and Stratagene.

The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification. These vectors

30 may be comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include plasmids, modified viruses, bacteriophage,

cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode a rhBRS-3 protein. It is well within the purview of the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

An expression vector containing DNA encoding a rhBRS-3 -like

5 protein may be used for expression of rhBRS-3 in a recombinant host cell. Such recombinant host cells can be cultured under suitable conditions to produce rhBRS-3 or a biologically equivalent form. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Commercially available mammalian expression vectors which may be

10 suitable for recombinant rhBRS-3 expression, include but are not limited to, pcDNA3.neo (Invitrogen), pcDNA3.1 (Invitrogen), pCI-neo (Promega), pLITMUS28, pLITMUS29, pLITMUS38 and pLITMUS39 (New England Biolabs), pcDNAI, pcDNAIamp (Invitrogen), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and lZD35 (ATCC 37565). Also, a variety of bacterial expression vectors may be used to express recombinant rhBRS-3 in bacterial cells. Commercially available bacterial expression vectors which may be suitable for recombinant rhBRS-3 expression

15 include, but are not limited to pCR2.1 (Invitrogen), pET11a (Novagen), lambda gt11 (Invitrogen), and pKK223-3 (Pharmacia). In addition, a variety of fungal cell expression vectors may be used to express recombinant rhBRS-3 in fungal cells. Commercially available fungal cell expression vectors which may be suitable for recombinant rhBRS-3 expression include but are not limited to pYES2 (Invitrogen)

20 and *Pichia* expression vector (Invitrogen). Also, a variety of insect cell expression vectors may be used to express recombinant protein in insect cells. Commercially available insect cell expression vectors which may be suitable for recombinant expression of rhBRS-3 include but are not limited to pBlueBacIII and pBlueBacHis2 (Invitrogen), and pAcG2T (Pharmingen).

25

30 The present invention, therefore, further relates to a process for expressing a rhesus monkey BRS-3 protein in a recombinant host cell, comprising:

(a) introducing a vector comprising an isolated nucleic acid molecule into a suitable host cell, the nucleic acid molecule comprising a sequence of nucleotides that encodes a rhesus monkey BRS-3 protein as set forth in SEQ ID NO:2; and, (b) culturing the host cell under conditions which allow expression of said rhesus monkey BRS-3 protein.

5 Expression of rhBRS-3 DNA may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

10 Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of bovine, porcine, monkey and rodent origin; and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. For instance, one insect expression system utilizes *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) in tandem with a baculovirus expression vector (pAcG2T, Pharmingen). Also, mammalian species which may be suitable and which 15 are commercially available, include but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), Saos-2 (ATCC HTB-85), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-25 C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171) and CPAE (ATCC CCL 209).

20 Following expression of rhBRS-3 in a host cell, rhBRS-3 protein may be recovered to provide rhBRS-3 protein in active form. Several rhBRS-3 protein purification procedures are available and suitable for use. Recombinant rhBRS-3 protein may be purified from cell lysates and extracts by various combinations of, or 30 individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and

hydrophobic interaction chromatography. In addition, recombinant rhBRS-3 protein can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full-length rhBRS-3 protein, or polypeptide fragments of rhBRS-3 protein.

5 Another preferred aspect of the present invention is a substantially purified membrane preparation, partially purified membrane preparation, or cell lysate which has been obtained from a recombinant host cell transformed or transfected with a DNA expression vector which comprises and appropriately expresses a complete open reading frame as set forth in SEQ ID NO:1, resulting in a 10 functional form of rhBRS-3. The subcellular membrane fractions and/or membrane-containing cell lysates from the recombinant host cells (both prokaryotic and eukaryotic as well as both stably and transiently transformed cells) contain the functional and processed proteins encoded by the nucleic acids of the present invention. This recombinant-based membrane preparation may comprise a rhesus 15 monkey BRS-3 protein and is essentially free from contaminating proteins, including but not limited to other rhesus monkey source proteins.

Therefore, a preferred aspect of the invention is a membrane preparation which contains a rhesus monkey BRS-3 comprising the functional form of the full length BRS-3 protein as disclosed in FIGURE 2 (SEQ ID NO:2). These 20 subcellular membrane fractions will comprise either wild type and/or mutant variations that are biologically functional forms of rhesus monkey BRS-3 at levels substantially above endogenous levels. Any such protein will be useful in various assays described throughout this specification to select for modulators of the rhBRS-3 protein. A preferred eukaryotic host cell of choice to express the rhBRS-3 molecules 25 of the present invention is a mammalian cell line, or *Xenopus* oocytes.

The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of rhBRS-3. The recombinant proteins, DNA molecules, RNA molecules and 30 antibodies lend themselves to the formulation of kits suitable for the detection and typing of rhBRS-3. Such a kit would comprise a compartmentalized carrier suitable

to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant rhBRS-3 or anti- rhBRS-3 antibodies suitable for detecting rhBRS-3. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like.

5 The assays described above can be carried out with cells that have been transiently or stably transfected with rhBRS-3. The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, protoplast fusion, and electroporation. Transfection is meant to include any method known in the art for introducing rhBRS-
10 3 into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing rhBRS-3, and electroporation. The expression vector-containing cells are individually analyzed to determine whether they produce rhBRS-3 protein. Identification of rhBRS-3 expressing cells may be done by several means, including
15 but not limited to immunological reactivity with anti- rhBRS-3 antibodies, and labeled ligand binding.

Human BRS-3 has been implicated in the regulation of neuroendocrine function and energy metabolism (Ohki *et al. Nature* 390: 165-69 (1997)). In addition, 20 mice lacking functional BRS-3 are hyperphagic and have a reduced metabolic rate, which leads to the development of obesity, hypertension and diabetes as adults. The present invention demonstrates that rhesus monkey and human BRS-3 have the same tissue-specific expression patterns (see EXAMPLE 3), and share high sequence similarity (see FIGURES 3 and 4), suggesting an involvement of rhesus monkey 25 BRS3 in energy homeostasis. These observations support the notion that rhesus monkey provides a good animal model to develop BRS-3 agonists as therapeutic agents for obesity.

Accordingly, the present invention is directed to methods for screening for compounds which modulate the expression of DNA or RNA encoding a rhBRS-3 30 protein as well as compounds which effect the function of the rhBRS-3 protein. Compounds that modulate these activities may be DNA, RNA, peptides, proteins, or

non-proteinaceous organic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding rhBRS-3, or the function of the rhBRS-3 protein. Compounds that modulate the expression of DNA or RNA encoding rhBRS-3 or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

One aspect of this portion of the present invention is a method for identifying compounds that modulate rhesus monkey bombesin receptor subtype-3 expression, comprising contacting a test compound with rhesus monkey bombesin receptor subtype-3, and determining whether the test compound interacts with rhesus monkey bombesin receptor subtype-3.

Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of rhBRS-3. For example, Cascieri et al. (1992, *Molec. Pharmacol.* 41:1096-1099) describe a method for identifying substances that inhibit agonist binding to rat neurokinin receptors and thus are potential agonists or antagonists of neurokinin receptors. The method involves transfecting COS cells with expression vectors containing rat neurokinin receptors, allowing the transfected cells to grow for a time sufficient to allow the neurokinin receptors to be expressed, harvesting the transfected cells and resuspending the cells in assay buffer containing a known radioactively labeled agonist of the neurokinin receptors either in the presence or the absence of the substance, and then measuring the binding of the radioactively labeled known agonist of the neurokinin receptor to the neurokinin receptor. If the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of the neurokinin receptor. Where binding of the substance such as an agonist or antagonist to rhBRS-3 is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, e.g., radioactively, fluorescently, enzymatically.

The specificity of binding of compounds having affinity for rhBRS-3 can be shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to rhBRS-3 or that inhibit the 5 binding of a known, radiolabeled ligand of rhBRS-3 (such as the synthetic peptide, [D-Tyr-betaAla-Phe-Nle] bombesin) to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for rhBRS-3. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled 10 compounds or that can be used as activators in functional assays. Compounds identified by the above method again are likely to be agonists or antagonists of rhBRS-3 and may be peptides, proteins, or non-proteinaceous organic molecules. As noted elsewhere in this specification, compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding rhBRS-3, or by acting as an 15 agonist or antagonist of the rhBRS-3 protein. Again, these compounds that modulate the expression of DNA or RNA encoding rhBRS-3 or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the 20 levels of expression or function in a standard sample.

Therefore, the present invention provides a method for determining whether a substance is capable of binding to rhesus monkey BRS-3 (rhBRS-3) comprising:

- (a) providing test cells by transfecting cells with an expression 25 vector that directs the expression of rhBRS-3 in the cells;
- (b) exposing the test cells to the substance;
- (c) measuring the amount of binding of the substance to rhBRS-3;

and,

- (d) comparing the amount of binding of the substance to rhBRS-3 30 in the test cells with the amount of binding of the substance to control cells that have not been transfected with rhBRS-3.

Also provided herein is a method of identifying a substance which modulates rhBRS-3 receptor activity, comprising: (a) combining a test substance in the presence and absence of a rhBRS-3 receptor protein wherein said rhBRS-3 receptor protein comprises the amino acid sequence as set forth in SEQ ID NO:2; 5 and, (b) measuring and comparing the effect of the test substance in the presence and absence of the rhBRS-3 receptor protein.

This invention further provides a method for determining whether a substance is a potential agonist or antagonist of rhBRS-3 comprising: (a) transfecting or transforming cells with an expression vector that directs expression of rhBRS-3 in 10 the cells, resulting in test cells; (b) allowing the test cells to grow for a time sufficient to allow rhBRS-3 to be expressed; (c) exposing the cells to a labeled ligand of rhBRS-3 in the presence and in the absence of the substance; and, (d) measuring the binding of the labeled ligand to rhBRS-3; where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the 15 substance, then the substance is a potential agonist or antagonist of rhBRS-3.

Pharmaceutically useful compositions comprising modulators of rhBRS-3 may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of 20 formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the protein, DNA, RNA, modified rhBRS-3, or either rhBRS-3 agonists or antagonists.

25 The present invention relates further to transgenic animals, either an invertebrate (e.g., *C. elegans*) or vertebrate (e.g., mouse), for which the gene encoding rhBRS-3 has been introduced into the germline of the animal. The purpose of this would be to inactivate, in the host, one or several endogenous BRS-3 and observe the biological effects. One such effect may well be an acquired resistance to 30 drugs that are agonists (activators) of BRS-3. In the case of drugs with suspected – but unproven – method of action (MOA) via BRS-3, such BRS-3-harboring transgenic animals may be used to confirm such an effect. Expression of the newly

introduced gene encoding rhBRS-3 into the host can be constitutive or inducible, depending on the type of promoter used to drive its expression. Also depending on the type of promoter used, expression of rhBRS-3 can be targeted to a given tissue(s) or it can be generalized.

5

All publications mentioned herein are incorporated by reference for the purpose of describing and disclosing methodologies and materials that might be used in connection with the present invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of 10 prior invention.

15

Having described preferred embodiments of the invention with reference to the accompanying drawings, it is to be understood that the invention is not limited to those precise embodiments, and that various changes and modifications may be effected therein by one skilled in the art without departing from the scope or 15 spirit of the invention as defined in the appended claims.

The following examples illustrate, but do not limit the invention.

EXAMPLE 1

Isolation of Rhesus Monkey BRS-3 cDNA

20

Poly(A)+ mRNA was isolated from rhesus monkey hypothalamus using the FastTrack® 2.0 mRNA isolation kit (Invitrogen, Carlsbad, CA). cDNAs were synthesized using SuperScript First-Strand Synthesis System (Invitrogen) with oligo (dT) priming. Using the cDNAs as templates, PCR reactions were carried out with the following primers designed from the 5' and 3' untranslated region of the

25

human BRS-3 sequence: BRS3-10 5' – T T G G A C G T G A C A A T C A C T G T A T T T G A A C T G A G A – 3' (SEQ ID NO:3) and BRS3-14 5' – T G T T T C T C C T C C C A G C A T G T G T A T C C G – 3' (SEQ ID NO:4). The resulting products were cloned into a pCR4-TOPO vector (Invitrogen). Seven clones were randomly selected from the independent reactions and sequenced using the dye- 30 terminator method with M13 forward and reverse primers as well as with the following oligonucleotide primers: P21 5' – G C T C T C T T T C A T C C G G C T C

– 3' (SEQ ID NO:5), P22 5' – C T G C C T T G T A T C T G T C A G C G – 3'
(SEQ ID NO:6), P23 5' – C A T G C C C G T A A G C A G G T T – 3' (SEQ ID
NO:7) P24 5' – C A G C A G A G G G C A A A C A G A G – 3' (SEQ ID NO:8).

One of the above clones, which has a sequence that is identical to the
5 consensus sequence generated from the seven rhesus clones, was chosen as a template
for PCR amplification using the following primers: BRS3kozak-F 5' – A T G G G A
T C C G C C A C C A T G G C T C A A A G G C A G C C T C A C T C A C C T –
3' (SEQ ID NO:9), BRS3Kozak-R 5' – A T G C T C G A G T G G A A A G C T A
G A C T C T A T C C T C T G C C T G C – 3' (SEQ ID NO:10). The amplified
10 products were cloned into the *Bam*HI and *Eco*RI sites of pcDNA3.1/Hyg (+)
(Invitrogen).

EXAMPLE 2

Poly(A)+mRNA isolation and cDNA synthesis

15 Poly(A)+mRNAs used in this study were isolated from rhesus monkey
hypothalamus, cerebellum, pituitary gland, pon, medulla oblongata, liver, brain
(except for hypothalamus, cerebellum, pituitary gland, pon, medulla oblongata), liver,
and testis using the FastTrack® 2.0 mRNA Isolation Kit (Invitrogen, Carlsbad, CA).
First strand cDNA was synthesized from 1 ug of mRNA in a 50 µl reaction volume
20 using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City,
CA). Reverse transcriptase reactions were also carried out without template to
determine if there was any genomic DNA contamination.

EXAMPLE 3

Analysis of BRS-3 Expression in Rhesus Monkey by Taq-Man PCR

25 The distribution of BRS-3 transcripts in adult rhesus monkey was
determined using real-time Taq-Man PCR. PCR primers and probes were designed
using Primer Express (Applied Biosystems). Primers and probe designed to detect
rhesus β-actin were based on the sequence that was originally obtained from rhesus
30 monkey genomic DNA. The following primers and probes were used in the real time
PCR: BRS3 primer 1: 5'- G A A A G A G A G C A C C T T A C A A C C A A T T -

3' (SEQ ID NO:11); BRS3 primer 2: 5'- C C A G T G G A T G C A A C C C A C T A -3' (SEQ ID NO:12); FAM-labeled BRS3 probe: 5'- T T C C G A A C A G C C A T C C T T C T G C A A G -3' (SEQ ID NO:13); β -actin primer 1: 5' - G C A A G C A G G A G T A T G A C G A G T C T -3' (SEQ ID NO:14); β -actin primer 2: 5' - A 5 A C T A A G T C A C A G T C C G C C T A G A A G -3' (SEQ ID NO:15); VIC-labeled β -actin probe: 5' - C C C T T C C A T C G T C C A C C G C A A A T -3' (SEQ ID NO:16). Each of the oligonucleotide fluorescent probes listed above were 3'-labeled with TAMRA (6-carboxytetramethylrhodamine).

Following reverse transcription, the resulting cDNA templates were 10 PCR-amplified in an ABI PRISM® 7700 Sequence Detection Systems Instrument according to the manufacturer's manuscripts (Applied Biosystems, Foster City, CA). PCR amplifications were performed in 50 μ l reaction volumes containing 0.5 μ l of cDNA template, 25 μ l of TaqManUniversal PCR Master Mix (Applied Biosystems), 900 nM of each BRS3 and β -actin primer and 250 nM of BRS3 and β -actin probes. 15 The cycling conditions consisted of an initial step of 50°C for 2 min (UNG incubation) followed by 95°C for 10 min (denaturation), and 40 cycles of 95°C for 15 sec (denaturation) and 60°C for 1 min (annealing/extension). Expression data were normalized to β -actin expression level.

Taq-man PCR results indicate that BRS3 mRNA was preferentially 20 expressed in the hypothalamus and testis of rhesus monkeys. Lower levels of expression were detected in other brain regions, including cerebellum, pituitary gland, pons and medulla oblongata. The expression pattern of BRS3 in rhesus monkey mimicked that of the human, suggesting that BRS3 might be involved in energy homeostasis and supporting the notion that rhesus monkey provides a good animal 25 model to develop BRS3 agonists as therapeutic agents for obesity.

EXAMPLE 4

Functional Expression of Rhesus Monkey BRS3 cDNA.
Measurement of rhesus monkey BRS3 receptor expression in the 30 aequorin-expressing stable reporter cell line 293-AEQ17 (Button *et al.*, 1993. *Cell*

Calcium 14: 663-671) was performed using a Lumineskan RT luminometer (Labsystems Inc., Gaithersburg, MD) controlled by custom software written for a Macintosh PowerPC 6100. 293-AEQ17 cells (8×10^5 cells plated 18 hours before transfection in a T75 flask) were transfected with 22 μ g of rhesus monkey BRS3 receptor plasmid DNA: 264 μ g lipofectamine.

Following approximately 40 hours of expression, the apo-aequorin in the cells was charged for 4 hours with coelenterazine (10 μ M) under reducing conditions (300 μ M reduced glutathione) in ECB buffer (140 mM NaCl, 20 mM KCl, 20 mM HEPES-NaOH [pH=7.4], 5 mM glucose, 1 mM MgCl₂, 1 mM CaCl₂, 0.1 mg/ml bovine serum albumin). The cells were harvested, washed once in ECB medium and resuspended to 500,000 cells/ml. 100 μ l of cell suspension (corresponding to 5×10^4 cells) was then injected into the test plate containing the BRS3 agonist peptide D-Tyr6-betaAla11-Phe13-Nle14]bombesin6-14, and the integrated light emission was recorded over 30 seconds, in 0.5-second units. 20 μ L of lysis buffer (0.1% final Triton X-100 concentration) was then injected and the integrated light emission recorded over 10 seconds, in 0.5-second units.

The "fractional response" values for each well were calculated by taking the ratio of the integrated response to the initial challenge to the total integrated luminescence including the Triton X-100 lysis response. The EC₅₀ value for activation of the rhesus monkey BRS3 receptor was 10 nM. Consistent with results obtained using human BRS-3 (Pradhan et al. Eur J Pharmacol 343: 275-87 (1998); Ryan et al. J Biol Chem 273: 13613-24 (1998)), the synthetic peptide dYB has a nanomolar high affinity to rhesus monkey BRS-3, demonstrating that rhesus monkey BRS-3 is a functional ortholog.

WHAT IS CLAIMED:

1. An isolated nucleic acid molecule, comprising a sequence of nucleotides that encodes a rhesus monkey BRS-3 protein as set forth in SEQ ID NO:2.
2. The isolated nucleic acid molecule of claim 1 wherein the nucleic acid is DNA.
3. The isolated nucleic acid molecule of claim 1 wherein the nucleic acid is mRNA.
4. The isolated nucleic acid molecule of claim 1 wherein the nucleic acid is cDNA.
5. The isolated nucleic acid molecule of claim 1 wherein the sequence of nucleotides comprises the sequence of nucleotides set forth in SEQ ID NO:1.
6. An expression vector comprising the nucleic acid molecule of claim 1.
7. A host cell comprising the vector of claim 6.
8. A subcellular membrane fraction obtained from the host cell of claim 7 which contains recombinant rhesus monkey BRS-3 protein.
9. A process for expressing a rhesus monkey BRS-3 protein in a recombinant host cell, comprising:
 - (a) introducing a vector comprising the nucleic acid of claim 1 into a suitable host cell; and,
 - (b) culturing the host cell under conditions which allow expression of said rhesus monkey BRS-3 protein.

10. An isolated and purified rhesus monkey BRS-3 polypeptide comprising a sequence of amino acids as set forth in SEQ ID NO:2.

11. A method for identifying compounds that modulate rhesus monkey bombesin receptor subtype-3 expression, comprising contacting a test compound with rhesus monkey bombesin receptor subtype-3, and determining whether the test compound interacts with rhesus monkey bombesin receptor subtype-3.

10 12. A method for determining whether a substance is capable of binding to rhesus monkey BRS-3 (rhBRS-3) comprising:

- 5 (a) providing test cells by transfecting cells with an expression vector that directs the expression of rhBRS-3 in the cells;
- (b) exposing the test cells to the substance;
- 15 (c) measuring the amount of binding of the substance to rhBRS-3, and,
- (d) comparing the amount of binding of the substance to rhBRS-3 in the test cells with the amount of binding of the substance to control cells that have not been transfected with rhBRS-3.

20 13. A method of identifying a substance which modulates rhBRS-3 receptor activity, comprising:

- 25 (a) combining a test substance in the presence and absence of a rhBRS-3 receptor protein wherein said rhBRS-3 receptor protein comprises the amino acid sequence as set forth in SEQ ID NO:2; and,
- (b) measuring and comparing the effect of the test substance in the presence and absence of the rhBRS-3 receptor protein.

30 14. A method for determining whether a substance is a potential agonist or antagonist of rhBRS-3 comprising:

- (a) transfecting or transforming cells with the expression vector of claim 6, resulting in test cells;
- (b) allowing the test cells to grow for a time sufficient to allow rhBRS-3 to be expressed;

(c) exposing the cells to a labeled ligand of rhBRS-3 in the presence and in the absence of the substance; and,

(d) measuring the binding of the labeled ligand to rhBRS-3; where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of rhBRS-3.

5

TITLE OF THE INVENTION

RHESUS MONKEY BOMBESIN RECEPTOR SUBTYPE-3 (BRS-3),
NUCLEOTIDES ENCODING SAME, AND USES THEREOF

5 ABSTRACT OF THE DISCLOSURE

A rhesus monkey bombesin receptor subtype-3 has been isolated, cloned and sequenced. This receptor is characteristic of the G-protein family of receptors. Isolated rhesus monkey bombesin receptor subtype-3 may be used to screen and identify novel bombesin receptor modulators that may contribute to the 10 regulation of endocrine processes, metabolism, or the cell cycle. Such compounds may be used in the treatment of conditions that result from deregulated expression of bombesin receptor subtype-3.

FIGURE 1. Nucleotide Sequence of Rhesus Monkey BRS-3

1	ATGGCTAAA	GGCAGCCTCA	CTCACCTAAT	CAGACTTTAA	TTTCAATCAC
51	AAATGACACA	GAATCAAGCT	CTGTGGTTTC	TAACGATAAC	ACAAATAAAG
101	GACGGAGCGG	GGACAACCTCT	CCAGGAATAG	AAGCATTGTG	TGCCATCTAT
151	ATTACTTATG	CTGTGATCAT	TTCAGTGGGC	ATCCTTGAA	ATGCTATTCT
201	CATCAAAGTC	TTTTCAAGA	CCAAATCCAT	GCAAACAGTT	CCAAATATTT
251	TCATCACCCAG	CCTGGCTTT	GGAGATCTT	TACTTCTGCT	AACTTGTGTC
301	CCAGTGATG	CAACCCACTA	CCTTGCAGAA	GGATGGCTGT	TCGGAAGAAT
351	TGGTTGTAAG	GTGCTCTCTT	TCATCCGGCT	CACTTCTGTT	GGTGTGTCAG
401	TGTTCACGTT	AACAATTCTC	AGCGCTGACA	GATACAAGGC	AGTTGTGAAG
451	CCACTTGAGC	GACAGCCCTC	CAATGCCATC	CTGAAAGACTT	GTATAAAAGC
501	TGGCTGCGTC	TGGATCGTGT	CTATGATATT	TGCTCTACCT	GAGGCTATAT
551	TTTCAAAATGT	ATATTCTTTT	CGAGATCCA	ACAAAAATGT	GACATTGAA
601	TCGTGTACCT	CTTATCCTGT	CTCTAAGAAG	CTCTTGCAAG	AAATACATTC
651	TCTGCTGTGC	TTCTTAGTGCT	TCTACATTAT	TCCACTCTCT	ATTATCTCTG
701	TCTATTATTCTC	TTTGATTGCT	AGGACCCTTT	ATAAAAGCAC	CCTGAACATA
751	CCTACTGAGG	AACAAGGCCA	TGCCCCTAAG	CAGATTGAAT	CCCGGAAGAG
801	AATTGCCAGA	ACGGTATTGG	TGTTGGTGGC	TCTGTTGCC	CTCTGCTGGT
851	TGCCAAATCA	CCTCCGTAC	CTCTACCAT	CATTCACTTC	TCAAACCTAT
901	GTAGACCCCT	CTGCCATGCA	TTTCATTTTC	ACCATTTCCT	CTCGGGTTCT
951	GGCTTTCAAGC	AATTCTGCG	TAAACCCCTT	TGCTCTCTAC	TGGCTGAGCA
1001	AAACCTTCCA	GAAGCATTAA	AAAGCTCACT	TGTTCTGTG	CAAGGCAGAG
1051	CAGCCTGAGC	CTCCTGTTGC	TGACACCTCT	CTTACCAACCC	TGGCTGTGAT
1101	GGGAAGGGTC	CCGGGCACGT	GGAACATGCA	GATGTCGAA	ATTAGTGTGA
1151	CCTCGTTCCC	TGGGTGTAGT	GTGAAGCAGG	CAGAGGATAG	AGTCTAG

FIGURE 2. Amino Acid Sequence of Rhesus Monkey BRS-3 Protein

1 MAQRQPHSPN QILISITNDT ESSSVVSNDN TNKGRSGDNS PGIEALCAIY
51 ITYAVIISVG ILGNAILIKV FFKTKSMQTV PNIFITSLAF GDLLLLLTCV
101 PVDATHYLAE GWLFGRIGCK VLSFIRLTSV GVSVFTLTL SADRYKAVVK
151 PLERQPSNAI LKTCIKAGCV WIVSMIFALP EAIFSNVYSF RDPNKNVTFE
201 SCTSYVPVSKK LLQEIHSLLC FLVFYIIPSL IISVYVSLIA RTLYKSTLNI
251 PTEEQGHARK QIESRKRIAR TVLVLVALFA LCWLPHLLY LYHSFTSQTY
301 VDPSAMHFIF TIFSRVLAFLS NSCVNPFLAY WLSKTFQKHF KAQLFCCKAE
351 QPEPPVADTS LTTLAVMGRV PGTGNMQMSE ISVTSFPGCS VKQAEDRV

FIGURE 3 ALIGNMENT OF BRS-3 NUCLEOTIDE SEQUENCES

	Section 1					
	(1) 1	10	20	30	40	52
hBRS3	(1) ATGGCTCAAAGGAGCCTCACTCACCTAATCAGACTTTAATTCAATCACAA					
ratBRS3	(1) ATGGCTCAAAGGAGCCTCACTCACCTAATCAGACTTTAATTCAATCACAA					
rhBRS3	(1) ATGGCTCAAAGGAGCCTCACTCACCTAATCAGACTTTAATTCAATCACAA					
Consensus	(1) ATGGCTCAAAGGAGCCTCACTCACCTAATCAGACTTTAATTCAATCACAA					
	Section 2					
	(53) 53	60	70	80	90	104
hBRS3	(53) ATGACACAGAAATCATCAAGCTCTGTGGTTCTAACGATAACACAAATAAAGG					
ratBRS3	(53) ATGACACAGAAACATCAAGCTCTGCCGTCTCCAACGATACTACACCTAAAGG					
rhBRS3	(53) ATGACACAGAAATCAAGCTCTGTGGTTCTAACGATAACACAAATAAAGG					
Consensus	(53) ATGACACAGAAATCAAGCTCTGTGGTTCTAACGATAACACAAATAAAGG					
	Section 3					
	(105) 105	110	120	130	140	156
hBRS3	(105) ATGGAGCAGGGACAACACTCTCCAGGAATAGAACGATTGTGTGCCATCTATATT					
ratBRS3	(105) ATGGAGCAGGGACAACACTCTCCAGGAATAGAACGACTGTGTGCCATCTATATC					
rhBRS3	(102) ACGGAGCAGGGACAACACTCTCCAGGAATAGAACGATTGTGTGCCATCTATATT					
Consensus	(105) ATGGAGCAGGGACAACACTCTCCAGGAATAGAACGATTGTGTGCCATCTATATT					
	Section 4					
	(157) 157	170	180	190		208
hBRS3	(157) ACTTATGCTGTGATCATTTCAAGCTGGCATCCTTGGAAATGCTATTGTGATCA					
ratBRS3	(157) ACTTATGCTGTGATCATTTCAAGCTGGCATCCTTGGAAATGCTATCTCATCA					
rhBRS3	(154) ACTTATGCTGTGATCATTTCAAGCTGGCATCCTTGGAAATGCTATTCTCATCA					
Consensus	(157) ACTTATGCTGTGATCATTTCAAGCTGGCATCCTTGGAAATGCTATTCTCATCA					
	Section 5					
	(209) 209	220	230	240	250	260
hBRS3	(209) AAGTCTTTTCAAGACCAAATCCATGCAAACAGTTCCAATATTTCATCAC					
ratBRS3	(209) AAGTCTTTTCAAGACTAAATCCATGCAAACAGTTCCAATATTTCATCAC					
rhBRS3	(206) AAGTCTTTTCAAGACCAAATCCATGCAAACAGTTCCAATATTTCATCAC					
Consensus	(209) AAGTCTTTTCAAGACCAAATCCATGCAAACAGTTCCAATATTTCATCAC					
	Section 6					
	(261) 261	270	280	290	300	312
hBRS3	(261) CAGCCTGGCTTTGGAGATCTTACTCTGCTAACCTGTGTGCCAGTGGAT					
ratBRS3	(261) CAGCCTGGCTTTGGAGATCTTACTCTGCTAACCTGTGTGCCAGTGGAT					
rhBRS3	(258) CAGCCTGGCTTTGGAGATCTTACTCTGCTAACCTGTGTGCCAGTGGAT					
Consensus	(261) CAGCCTGGCTTTGGAGATCTTACTCTGCTAACCTGTGTGCCAGTGGAT					
	Section 7					
	(313) 313	320	330	340	350	364
hBRS3	(313) GCAACTCACTACCTTGAGAAGGATGGCTGTTGGAAAGAATGGGTGTAAGG					
ratBRS3	(313) GCAACCCACTACCTGGAGAAGGATGGCTGTTGGAAAGAATGGGTGTAAGG					
rhBRS3	(310) GCAACCCACTACCTTGAGAAGGATGGCTGTTGGAAAGAATGGGTGTAAGG					
Consensus	(313) GCAACCCACTACCTTGAGAAGGATGGCTGTTGGAAAGAATGGGTGTAAGG					

FIGURE 3

						Section 8
	(365) 365	370	380	390	400	416
hBRS3	(365) TGCTCTCTTCATCCGGCTCACTTCTGTTGGTGTGTCAGTGTACATTAAC					
ratBRS3	(365) TGCTTCTCTTCATCCGGCTCACTTCTGTCGGTGTGTCAGTGTACGCTGAC					
rhBRS3	(362) TGCTCTCTTCATCCGGCTCACTTCTGTTGGTGTGTCAGTGTACGTTAAC					
Consensus	(365) TGCTCTCTTCATCCGGCTCACTTCTGTTGGTGTGTCAGTGTACGTTAAC					
						Section 9
	(417) 417	430	440	450		468
hBRS3	(417) AATTCTCAGCGCTGACAGATAACAAGGCACTTGTGAAGGCCACTTGAGCGACAG					
ratBRS3	(417) AATTCTCAGCGCTGACAGATAACAAGGCACTTGTGAAGGCCACTTGAGCGACAG					
rhBRS3	(414) AATTCTCAGCGCTGACAGATAACAAGGCACTTGTGAAGGCCACTTGAGCGACAG					
Consensus	(417) AATTCTCAGCGCTGACAGATAACAAGGCACTTGTGAAGGCCACTTGAGCGACAG					
						Section 10
	(469) 469	480	490	500	510	520
hBRS3	(469) CCCTCCAATGCCATCCTGAAGACTTGTGTAAAAGCTGGCTGCGCTGGATCG					
ratBRS3	(469) CCCTCCAATGCCATCTGAAGACCTGTGCCAAAGCTGGCTGGATCG					
rhBRS3	(466) CCCTCCAATGCCATCCTGAAGACTTGTGTAAAAGCTGGCTGCGCTGGATCG					
Consensus	(469) CCCTCCAATGCCATCCTGAAGACTTGTGTAAAAGCTGGCTGCGCTGGATCG					
						Section 11
	(521) 521	530	540	550	560	572
hBRS3	(521) TGTCTATGATATTGCTCTACCTGAGGCTATATTTCAAATGTATACACTTT					
ratBRS3	(521) TGGCTATGATATTGCTCTACAGAGGCTATATTCTCAAATGTATACACTTT					
rhBRS3	(518) TGTCTATGATATTGCTCTACCTGAGGCTATATTTCAAATGTATATTCTTT					
Consensus	(521) TGTCTATGATATTGCTCTACCTGAGGCTATATTTCAAATGTATACACTTT					
						Section 12
	(573) 573	580	590	600	610	624
hBRS3	(573) TCGAGATCCAATAAAATGACATTGAATCTGTACCTCTTATCCTGTC					
ratBRS3	(573) CCAAGATCCTAACAAACGTAACATTGAATCTGTAACTCCTAACCTATC					
rhBRS3	(570) TCGAGATCCAACAAAATGTGACATTGAATCTGTACCTCTTATCCTGTC					
Consensus	(573) TCGAGATCCAACAAAATGTGACATTGAATCATGTACCTCTTATCCTGTC					
						Section 13
	(625) 625	630	640	650	660	676
hBRS3	(625) TCTAAGAAGCTTTGCAAGAAATACTTCTCTGCTGTGCTTCTTAGTGTCT					
ratBRS3	(625) TCTAAGAAGCTTTGCAAGAAATACTTCTCTGCTGTGCTTCTTAGTGTCT					
rhBRS3	(622) TCTAAGAAGCTTTGCAAGAAATACTTCTCTGCTGTGCTTCTTAGTGTCT					
Consensus	(625) TCTAAGAAGCTTTGCAAGAAATACTTCTCTGCTGTGCTTCTTAGTGTCT					
						Section 14
	(677) 677	690	700	710		728
hBRS3	(677) ACATTATTCCACTCTCTATTATCTCTGTCTACTATTCCTGATTGCTAGGAC					
ratBRS3	(677) ACATTATTCCACTCTCTGATTATCTCTGTCTATTATTCCTGATTGCCAGGAC					
rhBRS3	(674) ACATTATTCCACTCTCTATTATCTCTGTCTATTATTCCTGATTGCTAGGAC					
Consensus	(677) ACATTATTCCACTCTCTATTATCTCTGTCTATTATTCCTGATTGCTAGGAC					

FIGURE 3

Section 15

(729) 729	740	750	760	770	780
hBRS3	(729) CCTTTACAAAAGCACCTGAAACATACTACTGAGGAACAAAGCCATGCCCGT				
ratBRS3	(729) TCTTTACAAAAGCACCTGAAACATACCGACTGAGGAACAAAGCCATGCCCGA				
rhBRS3	(726) CCTTTATAAAAGCACCTGAAACATACTACTGAGGAACAAAGCCATGCCCGT				
Consensus	(729) CCTTTACAAAAGCACCTGAAACATACTACTGAGGAACAAAGCCATGCCCGT				

Section 16

(781) 781	790	800	810	820	832
hBRS3	(781) AAGCAGATTGAATCCCGAAGAGAATTGCCAGAACGGTATTGGTGTGGTGG				
ratBRS3	(781) AAGCAGATTGAATCCCGAAGAGAATTGCCAACGGTACTGGTGTGGTGG				
rhBRS3	(778) AAGCAGATTGAATCCCGAAGAGAATTGCCAGAACGGTATTGGTGTGGTGG				
Consensus	(781) AAGCAGATTGAATCCCGAAGAGAATTGCCAGAACGGTATTGGTGTGGTGG				

Section 17

(833) 833	840	850	860	870	884
hBRS3	(833) CTCTGTTGCCCTCTGCTGGTTGCCAAATCACCTCCTGTACCTTACCATTC				
ratBRS3	(833) CTCTGTTCGCACTCTGCTGGTTGCCAAATCACCTCCTGTATCTTACATC				
rhBRS3	(830) CTCTGTTGCCCTCTGCTGGTTGCCAAATCACCTCCTGTACCTTACCATTC				
Consensus	(833) CTCTGTTGCCCTCTGCTGGTTGCCAAATCACCTCCTGTACCTTACCATTC				

Section 18

(885) 885	890	900	910	920	936
hBRS3	(885) ATTCACTTCTCAAACCTATGTAGACCCCTCTGCCATGCATTTCAATTTCA				
ratBRS3	(885) ATTCACTTATGAAAGCTACCGCAGAGCCTTCTGATTCCTTTCTGTC				
rhBRS3	(882) ATTCACTTCTCAAACCTATGTAGACCCCTCTGCCATGCATTTCAATTTCA				
Consensus	(885) ATTCACTTCTCAAACCTATGTAGACCCCTCTGCCATGCATTTCAATTTCA				

Section 19

(937) 937	950	960	970		988
hBRS3	(937) ATTTTCTCTCGGGTTTGGCTTCAGCAATTCTTGCCTAAACCCCTTTGCTC				
ratBRS3	(937) ATTTTCTCTCGGGTGTGGCTTCAGTAATTCTTGCCTAAACCCCTTTGCTC				
rhBRS3	(934) ATTTTCTCTCGGGTTCTGGCTTCAGCAATTCTTGCCTAAACCCCTTTGCTC				
Consensus	(937) ATTTTCTCTCGGGTTCTGGCTTCAGCAATTCTTGCCTAAACCCCTTTGCTC				

Section 20

(989) 989	1000	1010	1020	1030	1040
hBRS3	(989) TCTACTGGCTGAGCAAAAGCTTCCAGAACGATTTAAAGCTCAGTTGTTCTG				
ratBRS3	(989) TGTATTGGCTGAGCAAACCTTCCAGAACGATTTAAAGCTCAGCTCTGCTG				
rhBRS3	(986) TCTACTGGCTGAGCAAAACCTTCCAGAACGATTTAAAGCTCAGTTGTTCTG				
Consensus	(989) TCTACTGGCTGAGCAAAACCTTCCAGAACGATTTAAAGCTCAGTTGTTCTG				

Section 21

(1041) 1041	1050	1060	1070	1080	1092
hBRS3	(1041) TTGCAAGGCAGAGCCTGAGCCTCTGTTGCTGACACCTCTTACCA				
ratBRS3	(1041) CTTCAAGGCAGAGCAGCCTGACCTCTTGGTGTGACACCCCCCTTAACAA				
rhBRS3	(1038) TTGCAAGGCAGAGCAGCCTGAGCCTCTGTTGCTGACACCTCTTACCA				
Consensus	(1041) TTGCAAGGCAGAGCAGCCTGAGCCTCTGTTGCTGACACCTCTTACCA				

FIGURE 3

						Section 22
(1093) 1093	1100	1110	1120	1130	1144	
hBRS3 (1093)	CTGGCTGTGATGGGAACGGTCCCAGGCACTGGGAGCAACAGATGTCTGAAA					
ratBRS3 (1093)	CTCCTGATGGGCGGGGTCCAGCTACTGGGAGTACACACCTCTCTGAAA					
rhBRS3 (1090)	CTGGCTGTGATGGGAAGGGTCCCAGGCACTGGGAGCATGCAGATGTCTGAAA					
Consensus (1093)	CTGGCTGTGATGGGAAGGGTCCCAGGCACTGGGAGCATACAGATGTCTGAAA					
						Section 23
(1145) 1145	1150	1160	1170	1180	1196	
hBRS3 (1145)	TTAGTGTGACCTCGTTCACTGGGTGTAGTGTGAAGCAGGCAGAGGACAGATT					
ratBRS3 (1145)	TTAGCGTGACCTCGTTAGGGCAGTACTGCCAAGAAAGGAGAGGACAGATT					
rhBRS3 (1142)	TTAGTGTGACCTCGTTCCCTGGGTGTAGTGTGAAGCAGGCAGAGGATAGAGT					
Consensus (1145)	TTAGTGTGACCTCGTTCACTGGGTGTAGTGTGAAGCAGGCAGAGGACAGAGT					
						Section 24
(1197) 11900						
hBRS3 (1197)	CTAG (SEQ. ID NO: 17)					
ratBRS3 (1197)	TTAG (SEQ. ID NO: 18)					
rhBRS3 (1194)	CTAG (SEQ. ID NO: 19)					
Consensus (1197)	CTAG (SEQ. ID NO: 19)					

FIGURE 4 ALIGNMENT OF BRS-3 AMINO ACID SEQUENCES

						Section 1
	(1) 1	10	20	30	40	53
hBRS3	(1) MAQRQPHSPNQTLISITNDTE	SSSVVSDNTNK	GWSGDN	SPGIEALCAIY		
ratBRS3	(1) M Q QRQPHSPNQTLISITNDTE	SSSAVSDNTPK	GWSGDN	SPGIEALCAIY		
rhBRS3	(1) MAQRQPHSPNQTLISITNDTE	SSSVVSDNTNK	GRSGDN	SPGIEALCAIY		
Consensus	(1) MAQRQPHSPNQTLISITNDTE	SSSVVSDNTNK	GWSGDN	SPGIEALCAIY		
						Section 2
	(54) 54	60	70	80	90	106
hBRS3	(54) YAVIISVGILGNAILIKVFFKTKSMQTV	PNIFITS	LAFGD	LE	LE	TCVPV
ratBRS3	(54) YAVIISVGILGNAILIKVFFKTKSMQTV	PNIFITS	LAFGD	LE	LE	TCVPV
rhBRS3	(53) YAVIISVGILGNAILIKVFFKTKSMQTV	PNIFITS	LAFGD	LE	LE	TCVPV
Consensus	(54) YAVIISVGILGNAILIKVFFKTKSMQTV	PNIFITS	LAFGD	LE	LE	TCVPV
						Section 3
	(107) 107	120	130	140		159
hBRS3	(107) HYLAEGWLFGRIGCKVLS	FI	RTS	VGVS	VFTL	TILSADRYKAVVVKPLERQPSN
ratBRS3	(107) HYLAEGWLFGRIGCKVLS	FI	RTS	VGVS	VFTL	TILSADRYKAVVVKPLERQPSN
rhBRS3	(106) HYLAEGWLFGRIGCKVLS	FI	RTS	VGVS	VFTL	TILSADRYKAVVVKPLERQPSN
Consensus	(107) HYLAEGWLFGRIGCKVLS	FI	RTS	VGVS	VFTL	TILSADRYKAVVVKPLERQPSN
						Section 4
	(160) 160	170	180	190	200	212
hBRS3	(160) AILKTC I KAGCVWIVSMIFALPEAIFSNVY	T	FRDP	PNKN	TFES	CTSY
ratBRS3	(160) AILKTC I KAGCVWIVSMIFALPEAIFSNVY	T	FRDP	PNKN	TFES	CTSY
rhBRS3	(159) AILKTC I KAGCVWIVSMIFALPEAIFSNVY	T	FRDP	PNKN	TFES	CTSY
Consensus	(160) AILKTC I KAGCVWIVSMIFALPEAIFSNVY	T	FRDP	PNKN	TFES	CTSY
						Section 5
	(213) 213	220	230	240	250	265
hBRS3	(213) LQEIHSSL	CFLVFYI	IPLSIISVY	YSLIARTLY	KSTLN	IPTEEQSHARKQIES
ratBRS3	(213) LQEIHSSL	CFLVFYI	IPLSIISVY	YSLIARTLY	KSTLN	IPTEEQSHARKQIES
rhBRS3	(212) LQEIHSSL	CFLVFYI	IPLSIISVY	YSLIARTLY	KSTLN	IPTEEQSHARKQIES
Consensus	(213) LQEIHSSL	CFLVFYI	IPLSIISVY	YSLIARTLY	KSTLN	IPTEEQSHARKQIES
						Section 6
	(266) 266	280	290	300		318
hBRS3	(266) RKRIARTV	LVLVALF	ALCWLPN	HLLY	HSFTSQ	TYVDP
ratBRS3	(266) RKRIARTV	LVLVALF	ALCWLPN	HLLY	HSFTSQ	TYVDP
rhBRS3	(265) RKRIARTV	LVLVALF	ALCWLPN	HLLY	HSFTSQ	TYVDP
Consensus	(266) RKRIARTV	LVLVALF	ALCWLPN	HLLY	HSFTSQ	TYVDP
						Section 7
	(319) 319	330	340	350	360	371
hBRS3	(319) AFSNSCVNP	FALY	WLSK	FQKHF	KAQLF	CCKAER
ratBRS3	(319) AFSNSCVNP	FALY	WLSK	FQKHF	KAQLC	CCFKAEQ
rhBRS3	(318) AFSNSCVNP	FALY	WLSK	FQKHF	KAQLF	CCKAEQ
Consensus	(319) AFSNSCVNP	FALY	WLSK	FQKHF	KAQLF	CCKAEQ

FIGURE 4

Section 8

(372) 372	380	399
hBRS3 (372) PGTGS E QMSEISVTSF G GCSVVKQAEDRF		(SEQ. ID NO: 20)
ratBRS3 (372) P T TGS AH SEISVTLF G S E AKK G ED M V		(SEQ. ID NO: 21)
rhBRS3 (371) PGTGN M QMSEISVTSFPGCSVVKQAEDRV		(SEQ. ID NO: 2)
Consensus (372) PGTGSIQMSEISVTSFSGCSVVKQAEDRV		(SEQ. ID NO: 22)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER: _____**

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.